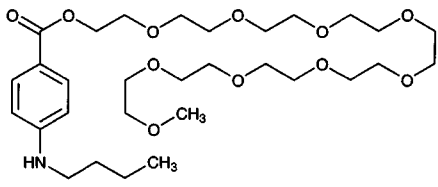
Benzonatate



Molecular formula: $C_{30}H_{53}NO_{11}$
Molecular weight: 603.75
CAS Registry No.: 104-31-4
Merck Index: 1127

SAMPLE

Matrix: tissue
Sample preparation: Homogenize 10 g tissue in 40 mL water, make alkaline with NaOH, extract twice with ether. Combine extracts and evaporate them to dryness, reconstitute the residue in 1 mL EtOH, inject a 50 µL aliquot.

HPLC VARIABLES

Column: Reverse phase ODS
Mobile phase: MeCN:100 mM phosphoric acid 50:50
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: UV 205

CHROMATOGRAM

Limit of detection: 200 ng/mL

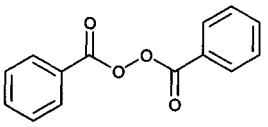
KEY WORDS

liver; brain; blood; kidney

REFERENCE

Cohan,J.A.; Manning,T.J.; Lukash,L.; Long,C.; Ziminski,K.R.; Conradi,S.E. Two fatalities resulting from Tessalon (benzonatate), *Vet.Hum.Toxicol.*, **1986**, *28*, 543–544.

Benzoyl peroxide



Molecular formula: $C_{14}H_{10}O_4$
Molecular weight: 242.23
CAS Registry No.: 94-36-0
Merck Index: 1149

SAMPLE

Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C18 SPE cartridge with 2 mL MeCN and 2 mL 50 mM pH 7.5 phosphate buffer. Add 1 mL serum to the SPE cartridge, wash with 500 µL 50 mM pH 7.5 phosphate buffer, elute with 1 mL MeCN, inject a 20 µL aliquot of the eluate.

HPLC VARIABLES

Column: Capcell Pak C-18 SG-120 (Shiseido)
Mobile phase: MeCN:water 50:50
Flow rate: 1.2
Injection volume: 20
Detector: UV 235

CHROMATOGRAM

Retention time: 24

OTHER SUBSTANCES

Extracted: methyl methacrylate, N,N-dimethyl-p-toluidine

KEY WORDS

serum; dental material; methyl methacrylate polymer; plexiglass; horse; SPE

REFERENCE

Shintani,H.; Tsuchiya,T.; Hata,Y.; Nakamura,A. Solid phase extraction and HPLC analysis of toxic components eluted from methyl methacrylate dental materials, *J.Anal.Toxicol.*, **1993**, *17*, 73–78.

SAMPLE

Matrix: food
Sample preparation: Mix 1 g flour with 8.0 mL EtOH, shake vigorously for 1 min and extract by sonication for 15 min at room temperature. Centrifuge at 4000 rpm for 20 min, remove 4 mL supernatant, add 2 mL 100 mM KOH, mix in an ultrasonic water bath for 2 min at room temperature, dilute to 25 mL with water and set aside overnight. Filter (0.45 µm) and inject a 50 µL aliquot.

HPLC VARIABLES

Column: Dionex OmniPac PAX-100 anion exchange column
Mobile phase: MeOH:3 mM sodium carbonate 2:98
Flow rate: 1.0
Injection volume: 50
Detector: UV 222

CHROMATOGRAM

Retention time: 2.5
Limit of detection: 19 ng/mL
Limit of quantitation: 120 ng/mL

OTHER SUBSTANCES
Interfering: benzoic acid

KEY WORDS
wheat flour; derivatization

REFERENCE
Chen,Q.-C.; Mou,S.-F.; Hou,X.-P.; Ni,Z.-M. Determination of benzoyl peroxide in wheat flour by ion chromatography with precolumn derivatization, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 705–716.

SAMPLE
Matrix: plastic
Sample preparation: Leach with 20 mL water, MeOH, acetone, or THF at room temperature for 24 h, repeat. Combine solutions and add an equal volume of MeCN:water 50:50, filter (0.45 μm) the supernatant, inject a 20 μl aliquot.

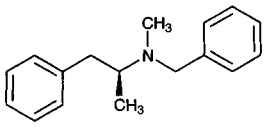
HPLC VARIABLES
Column: 250 × 4.6 Capcell Pak SG-120 C18 (Shiseido)
Mobile phase: MeCN:water 48:52
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 29.6

OTHER SUBSTANCES
Simultaneous: methyl methacrylate, N,N-dimethyl-p-toluidine

REFERENCE
Shintani,H. HPLC analysis of toxic additives and residual monomer from dental plate, *J.Liq.Chromatogr.*, **1995**, *18*, 613–626.

Benzphetamine



Molecular formula: $C_{17}H_{21}N$
Molecular weight: 239.36
CAS Registry No.: 156-08-1, 5411-22-3 (HCl)
Merck Index: 1151
Lednicer No.: 1 70

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 1.52

OTHER SUBSTANCES

Simultaneous: phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: pemoline, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine

REFERENCE

Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.9

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

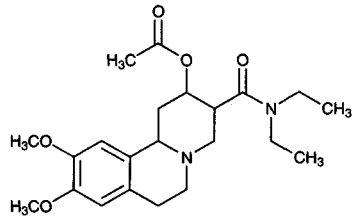
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Benzquinamide



Molecular formula: $C_{22}H_{32}N_2O_5$
Molecular weight: 404.51
CAS Registry No.: 63-12-7
Merck Index: 1154
Lednicer No.: 1 350

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.2

OTHER SUBSTANCES

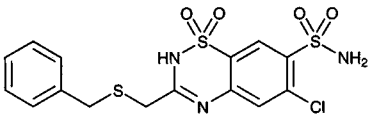
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl,

protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Benzthiazide



Molecular formula: $C_{15}H_{14}ClN_3O_4S_3$
Molecular weight: 431.94
CAS Registry No.: 91-33-8
Merck Index: 1155

SAMPLE

Matrix: blood, feces, urine

Sample preparation: Plasma. 3 mL Plasma + 500 µL 1 µg/mL polythiazide in 10 mM NaOH + 1 mL 10 mM NaOH + 800 µL 100 mM HCl + 10 mL dichloromethane, shake on a platform shaker for 20 min, centrifuge at -10° at 3000 rpm for 15 min, repeat extraction twice more. Combine all organic layers and evaporate them to dryness under a stream of nitrogen at 50°, reconstitute the residue in 50 µL, vortex, inject whole amount. Urine. 5 mL Urine + 1 mL 2 µg/mL polythiazide in 10 mM NaOH + 1 mL 10 mM NaOH + 2 mL 0.68% KH_2PO_4 adjusted to pH 6.1 + 10 mL dichloromethane, shake on a platform shaker for 20 min, centrifuge at -10° at 3000 rpm for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 µL, vortex, inject a 20 µL aliquot. Feces. Extract 20 g feces with 350 mL acetone, filter (Whatman No. 1 paper), evaporate to ca. 50 mL, make up to 100 mL with acetone. Remove a 10 mL aliquot and add it to 1 mL 300 µg/mL polythiazide in acetone, evaporate to dryness under nitrogen, dissolve in 10 mL MeOH, inject a 10 µL aliquot.

HPLC VARIABLES

Column: µBondapak C18

Mobile phase: MeCN:glacial acetic acid:water 35:2:63 (plasma, feces) or MeCN:water 40:60 (urine)

Flow rate: 2

Injection volume: 10-50

Detector: UV 280

CHROMATOGRAM

Retention time: 7 (plasma), 5 (urine)

Internal standard: polythiazide (9 (plasma), 8 (urine))

Limit of detection: 10 ng/mL (plasma)

Limit of quantitation: 50 ng/mL (urine), 20 ng/mL (plasma)

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Meyer,M.C.; Hwang,P.; Straughn,A.B.; Rotenberg,K. HPLC determination of benzthiazide in biologic material, *Biopharm.Drug Dispos.*, **1982**, *3*, 1–9.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: not specified

Mobile phase: MeOH:water containing 200 mM ammonia and 200 mM glycolic acid 25:75

Flow rate: 0.8

Detector: MS, Hewlett-Packard 9000-300 quadrupole, thermospray, stem 108-115°, tip 180-205°, ion source 276°, filament on, negative ion mode

KEY WORDS

LC-MS

REFERENCE

Kim,Y.; Park,S.; Park,J.; Lee,W. Detection of benzthiazide by high-performance liquid chromatography-thermospray mass spectrometry, *J.Chromatogr.A*, **1995**, *689*, 170–174.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)

Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 5

Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 13.5 (A), 14.3 (B)

Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, cyclothiazide, polythiazide, bendroflumethiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, canrenone, flumethiazide

Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE

Matrix: urine

Sample preparation: Make 5 mL urine alkaline (pH 9-10), add 2 g NaCl, extract twice with 6 mL ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN/water, inject a 10-20 μL aliquot.

HPLC VARIABLES

Column: 100 × 4 5 μm SGE 100 GL-4 C18P (Scientific Glass Engineering)

Mobile phase: MeCN:MeOH:water:trifluoroacetic acid 4.5:10.5:85:0.5

Flow rate: 0.8 or 1

Injection volume: 10-20

Detector: MS, ZAB2-SEQ (VG), PSP source coupled to LC, source 250°, probe 240-260°, scan m/z 200-550 or UV 270

CHROMATOGRAM
Retention time: 7.2
Limit of detection: 50 ng (by MS)

OTHER SUBSTANCES
Extracted: amiloride, chlorthalidone, triamterene, furosemide, bendroflumethiazide

REFERENCE
Ventura,R.; Fraisse,D.; Becchi,M.; Paisse,O.; Segura,J. Approach to the analysis of diuretics and masking agents by high-performance liquid chromatography-mass spectrometry in doping control, *J.Chromatogr.*, **1991**, *562*, 723–736.

SAMPLE
Matrix: urine
Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 μL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES
Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18
Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)
Flow rate: 1
Injection volume: 40
Detector: UV 230

CHROMATOGRAM
Retention time: 17.6
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: acetazolamide, amiloride, bendroflumethiazide, bumetanide, caffeine, carbamazepine, chlorothiazide, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, furosemide, hydrochlorothiazide, metyrapone, probenecid, spironolactone, triamterene, trichlormethiazide

KEY WORDS
column-switching; optimum detection wavelengths vary for each drug

REFERENCE
Saarinen,M.; Sirén,H.; Riekkola,M.-L. A column switching technique for the screening of diuretics in urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4063–4078.

SAMPLE
Matrix: urine
Sample preparation: 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN:water 15:85 and inject 20 μL aliquots. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES
Column: 75 × 4.6 3 μm Ultrasphere ODS

Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid. From 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min. Kept at 60:40 for 1 min, decreased to 10:90 over 1 min and equilibrated at 10:90 for 2 min.
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: 6.5
Internal standard: 7-propyltheophylline (4.5)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES

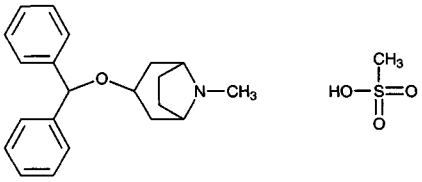
Simultaneous: xipamide, bumetanide, acetazolamide, amiloride, bendroflumethiazide, buthiazide, caffeine, canrenone, chlorthalidone, clopamide, cyclothiazide, diclofenamide, ethacrynic acid, furosemide, hydrochlorothiazide, mesocarb, morazone, piretanide, polythiazide, probenecid, spironolactone, torsemide, triamterene

REFERENCE

Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

Benztropine mesylate

Molecular formula: $C_{22}H_{29}NO_4S$
Molecular weight: 403.54
CAS Registry No.: 132-17-2, 86-13-5 (free base)
Merck Index: 1156



SAMPLE

Matrix: blood

Sample preparation: 10 mL Plasma or whole blood + 1 mL 1 M NaOH, extract twice with 10 mL hexane for 30 min. Remove the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 100 mM HCl, add 5 mL chloroform, vortex for 1 min, centrifuge. Remove a 4.5 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. (It is implied, but not explicitly stated in the paper, that this extraction procedure works for this compound.)

HPLC VARIABLES

Column: 10 μm Micropak CN (Varian)
Mobile phase: MeCN:20 mM ammonium acetate 90:10
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 13.2
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: acetophenazine, amitriptyline, butaperazine, carphenazine, chlorpromazine, fluphenazine, haloperidol, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, promazine, promethazine, thioridazine, thiothixene, trifluoperazine, triflupromazine, trihexyphenidyl, trimeprazine

KEY WORDS

plasma; whole blood

REFERENCE

Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 150 μL 200 ng/mL desipramine hydrochloride + 150 μL 5 M NaOH, vortex, add 1 mL ethylene glycol, vortex, add 10 mL hexane, shake on a rotary shaker at 30 rpm for 30 min, centrifuge at 1000 g at 4°. Remove the organic layer and add it to 300 μL 100 mM HCl, shake at high speed for 20 min, centrifuge, inject a 200 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 150 × 3.9 5 μm Spherisorb C8

Mobile phase: MeCN:buffer 60:40 (Buffer was 1.5 mL triethylamine in 1 L water adjusted to pH 3.0 with 85% phosphoric acid.)
Flow rate: 1.5
Injection volume: 200
Detector: UV 199

CHROMATOGRAM

Retention time: 7.0
Internal standard: desipramine hydrochloride (5.3)
Limit of quantitation: 0.25 ng/mL

OTHER SUBSTANCES

Simultaneous: hyoscyamine, orphenadrine, bromocriptine, biperiden
Noninterfering: amantadine, carbidopa, levodopa

KEY WORDS

plasma

REFERENCE

Selinger,K.; Lebel,G.; Hill,H.M.; Discenza,C. High-performance liquid chromatographic method for the analysis of benztropine in human plasma, *J.Chromatogr.*, **1989**, *491*, 248–252.

SAMPLE

Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 22.75
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES

Simultaneous: amoxapine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexiphenidyl, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine
Interfering: promethazine, trimipramine, amitriptyline

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 2.10

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

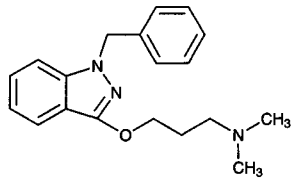
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenpro-

porex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Benzydamine



Molecular formula: $C_{19}H_{23}N_3O$
Molecular weight: 309.41
CAS Registry No.: 642-72-8, 132-69-4 (HCl)
Merck Index: 1157
Lednicer No.: 1 323

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 215.8

CHROMATOGRAM

Retention time: 14.955

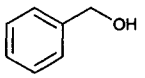
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Benzyl alcohol



Molecular formula: C_7H_8O
Molecular weight: 108.14
CAS Registry No.: 100-51-6
Merck Index: 1159

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 600 μg 7-[(imidazolemethanoyl)methoxy]-4-methylcoumarin in 2.5 mL toluene and 750 μL MeCN, add 20 μL benzyl alcohol, add 150 μL 93.3 μg/mL 4-dimethylaminopyridine in MeCN, mix vigorously, heat at 60° for 1.5 h, cool, evaporate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 300 μL mobile phase, inject a 5 μL aliquot. (Preparation of 7-[(imidazolemethanoyl)methoxy]-4-methylcoumarin is as follows. Stir 102.3 mg 7-(carboxymethoxy)-4-methylcoumarin in 7 mL THF, add 70.9 mg 1,1'-carbonyldiimidazole in one portion, reflux for 30 min, stir at room temperature for 5 h. Filter and dry the solid under reduced pressure to obtain 7-[(imidazolemethanoyl)methoxy]-4-methylcoumarin as a white solid (mp 161-162°). Fluorescence detection can also be used.)

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeCN:MeOH:100 mM pH 5.5 ammonium acetate buffer 50:1.5:48.5
Flow rate: 0.7
Injection volume: 5
Detector: MS, Hewlett-Packard 5989A, thermospray interface, filament-assisted ionization mode, ion source 280°, probe stem 112°, probe tip 235-245°

CHROMATOGRAM

Retention time: 7
Limit of detection: 0.8 ng

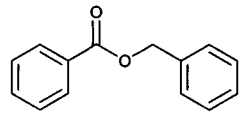
KEY WORDS

derivatization

REFERENCE

Phillips,L.R.; Supko,J.G.; Wolfe,T.L.; Malspeis,L. Precolumn derivatization of hydroxy compounds with 7-[(imidazolemethanoyl)methoxy]-4-methylcoumarin (IMMC) and LC/TSP-MS of the resulting esters, *Proc.Am.Soc.Mass Spectrom.*, **1995**, *43*, 163–164.

Benzyl benzoate



Molecular formula: $C_{14}H_{12}O_2$
Molecular weight: 212.25
CAS Registry No.: 120-51-4
Merck Index: 1162

SAMPLE

Matrix: formulations
Sample preparation: Dilute 0.5 mL of nanocapsules suspension 1:200 with MeCN, filter, inject an aliquot. Alternatively, evaporate 5 mL of a nanocapsule suspension to dryness and dissolve the residue in 150 mL dichloromethane or ethyl acetate, dry over anhydrous sodium sulfate. Evaporate to dryness under reduced pressure, take up the residue in 50 mL MeOH, dilute 1:20 with MeOH, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm Nucleosil C18
Mobile phase: MeCN: water 75:25
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 4.71

OTHER SUBSTANCES

Simultaneous: progesterone
Noninterfering: poly-ϵ-caprolactone

KEY WORDS

nanocapsules

REFERENCE

Benali,S.; Tharasse-Bloch,C.; André; Vérité,P.; Duclos,R.; Lafont,O. Determination od progesterone in nanocapsules by high performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3233–3243.

SAMPLE

Matrix: formulations
Sample preparation: Injections. Extract 2 mL with EtOH:water 85:15, make up extracts to 100 mL with EtOH:water 85:15, remove a 2 mL aliquot and add it to 1 mL 1 mg/mL hydrocortisone in EtOH. Dilute this mixture to 50 mL with EtOH:water 50:50, inject an aliquot. Suspensions. Dilute 2 mL suspension to 100 mL with EtOH, filter (if necessary), remove a 2 mL aliquot and add it to 1 mL 1 mg/mL hydrocortisone in EtOH. Dilute this mixture to 50 mL with EtOH, inject an aliquot.

HPLC VARIABLES

Column: 300 × 4 μBondapak CN
Mobile phase: MeOH:20 mM KH_2PO_4 30:70
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 6.5

Internal standard: hydrocortisone (2.5)

OTHER SUBSTANCES
Simultaneous: medroxyprogesterone acetate
Noninterfering: polyethylene glycol 4000, mystristyl-gamma-picolinium chloride, methylcellulose, thimerosal
Interfering: progesterone

REFERENCE
Das Gupta,V. Quantitation of hydroxyprogesterone caproate, medroxyprogesterone acetate, and progesterone by reversed-phase high-pressure liquid chromatography, *J.Pharm.Sci.*, **1982**, *71*, 294–297.

SAMPLE
Matrix: formulations
Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 11.8
Limit of detection: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: aspirin, caffeine, formebolone, benzyl alcohol, testolactone, cortisone, fluoxymesterone, norethindrone, oxandrolone (UV 210), boldenone, ethisterone, methandrostenolone, nandrolone, norgestrel, testosterone, dehydroepiandrosterone (UV 210), miborelone, methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, trenbolone acetate, nandrolone acetate, testosterone acetate, stanozolol, oxymetholone, nandrolone propionate, methenolone acetate, testosterone propionate

KEY WORDS
oils; tablets; suspensions

REFERENCE
Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 50 mg formulation, add 5 mL 1.5 mg/mL benzophenone in MeOH, make up to 50 mL with MeOH. Dilute 1 mL of this solution to 10 mL with MeOH, filter (0.45 μm PTFE membrane), inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 30 mm long Brownlee guard column

Column: 220 × 4.6 5 μm C18 (Brownlee)
Mobile phase: MeCN:water 60:40
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5
Internal standard: benzophenone (3.3)
Limit of quantitation: 7000 ng/mL

OTHER SUBSTANCES
Simultaneous: benzocaine

KEY WORDS
dermatological preparations

REFERENCE
Gigante,B.; Barros,A.M.V.; Teixeira,A.; Marcelo-Curto,M.J. Separation and simultaneous high-performance liquid chromatographic determination of benzocaine and benzyl benzoate in a pharmaceutical preparation, *J.Chromatogr.*, **1991**, *549*, 217–220.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 100 μg/mL, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 CO:Pell ODS
Column: 300 × 3.9 Bondex C18 (Phenomenex)
Mobile phase: MeOH:water 85:15
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Also analyzed: boldenone, testosterone, nandrolone, and their esters

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R.; DeRuiter,J. Liquid chromatographic and mass spectral analysis of the anabolic 17-hydroxy steroid esters, *J.Chromatogr.Sci.*, **1990**, *28*, 263–268.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.9 5 μm Zorbax BP-ODS
Mobile phase: MeCN:50 mM pH 7.2 sodium phosphate buffer 40:60
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8

Internal standard: benzyl benzoate

OTHER SUBSTANCES

Extracted: HO-221

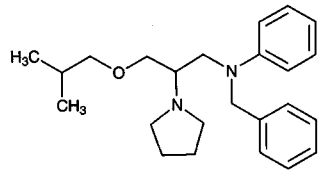
KEY WORDS

benzyl benzoate is IS

REFERENCE

Kondo,N.; Iwao,T.; Hirai,K.-I.; Fukuda,M.; Yamanouchi,K.; Yokoyama,K.; Miyaji,M.; Ishihara,Y.; Kon,K.; Ogawa,Y.; Mayumi,T. Improved oral absorption of enteric coprecipitates of a poorly soluble drug, *J.Pharm.Sci.*, **1994**, *83*, 566–570.

Bepridil



Molecular formula: $C_{24}H_{34}N_2O$
Molecular weight: 366.55
CAS Registry No.: 64706-54-3, 74764-40-2 (HCl monohydrate)
Merck Index: 1188
Lednicer No.: 3 46

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 249

CHROMATOGRAM

Retention time: 18.30
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 202.8

CHROMATOGRAM

Retention time: 19.503

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations

Sample preparation: Weigh out ground tablets or capsules equivalent to 150 mg bepridil.HCl, add 150 mL MeCN, shake for 30 min, dilute to 200 mL with MeCN, filter (Schleicher & Schüll paper, grade 588), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4.6 10 μm μBondapak C18
Mobile phase: MeCN:buffer 580:405 (Buffer was 1.1 g sodium 1-heptanesulfonate in 405 mL water, adjust to pH 2.37 with glacial acetic acid (ca. 15 mL).)
Column temperature: 35
Flow rate: 1.3
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.8

OTHER SUBSTANCES
Simultaneous: impurities, benzaldehyde, N-benzylaniline, benzoic acid

KEY WORDS
stability-indicating; rugged; capsules; tablets

REFERENCE
Renzi,N.L.; Fronheiser,M.E.; Duong,H.T.; Fulton,D.J.; Rabinowitz,M. Stability-indicating high-performance liquid chromatography assay for bepridil hydrochloride drug substance and drug products, *J.Chromatogr.*, **1989**, *462*, 398–405.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex 3014 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 62:35:3 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 250

KEY WORDS
chiral; α = 1.22 for enantiomers

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 100 μM solution in buffer, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 column containing riboflavin binding proteins (Prepare as follows. Add riboflavin to saturate protein of egg yolk, homogenize with 3 volumes buffer, centrifuge, add the supernatant to a 500 × 30 column of DEAE-cellulose (Whatman) equilibrated with buffer, wash extensively with buffer to remove bound protein, elute riboflavin binding proteins (RFBP) with buffer containing 200 mM NaCl (RFBP has intense yellow color, absorption at 455 nm). Purify RFBP on a Sephadex G-100 column with 50 mM pH 7.5 Tris-HCl buffer as eluent, remove the bound riboflavin by extensive dialysis at pH 3.0. Add 4.5 g N,N-disuccinylimidyl carbonate to 3 g Nucleosil 5NH2 slurried in MeCN, filter, wash with MeCN, wash with 50 mM pH 7.5 phosphate buffer. Suspend 300 mg RFBP in 50 mM phosphate buffer, add the activated silica, mix gently for 2 h using a rotary evaporator, filter, wash with sterile water, wash with isopropanol:water 1:2, pack in a 100 × 4.6 column.) (Buffer was 100 mM pH 5.3 sodium acetate.)

Mobile phase: EtOH:50 mM pH 5.5 KH_2PO_4 5:95
Flow rate: 0.8
Injection volume: 20
Detector: UV

CHROMATOGRAM

Retention time: k' 11.96

OTHER SUBSTANCES

Simultaneous: lorazepam, manidipine, nicardipine, oxazepam

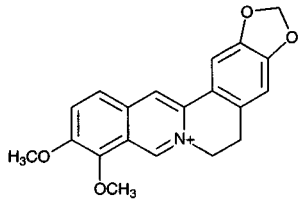
KEY WORDS

chiral; α = 1.21

REFERENCE

Massolini,G.; De Lorenzi,E.; Ponci,M.C.; Gandini,C.; Caccialanza,G.; Monaco,H.L. Egg yolk riboflavin binding protein as a new chiral stationary phase in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *704*, 55–65.

Berberine



Molecular formula: $C_{20}H_{18}NO_4$
Molecular weight: 336.37
CAS Registry No.: 2086-83-1
Merck Index: 1192

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

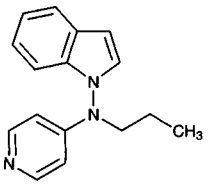
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, py-

rilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Besipirdine



Molecular formula: $C_{16}H_{17}N_3$
Molecular weight: 251.33
CAS Registry No.: 119257-34-0, 130953-69-4 (HCl), 119257-40-8 (maleate)
Merck Index: 1223

SAMPLE

Matrix: cell cultures
Sample preparation: Mix 1 mL cell culture with 50 μL 1 mg/mL IS in MeOH and 1 mL saturated aqueous sodium bicarbonate, add 3 mL ethyl acetate:cyclohexane 50:50, invert at 18 cycles/min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 2 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 2μm Hypersil-Phenyl
Mobile phase: MeCN:buffer 85:15 (Buffer was 5.8 mM triethylammonium formate adjusted to pH 2.5 with 90% formic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: 10
Internal standard: 3-ethyl-N-methyl-N-(4-pyridinyl)-1H-indol-1-amine hydrochloride

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

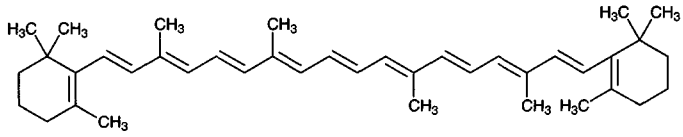
details of semipreparative HPLC

REFERENCE

Rao,G.P.; Davis,P.J. Microbial models of mammalian metabolism. Biotransformations of HP 749 (besperidine) using Cunninghamella elegans, *Drug Metab.Dispos.*, **1997**, *25*, 709–715.

Beta-carotene

Molecular formula: $C_{20}H_{56}$
Molecular weight: 536.88
CAS Registry No.: 7235-40-7
Merck Index: 1902



SAMPLE

Matrix: blood

Sample preparation: Centrifuge 200 µL serum, add 200 µL IS and 200 µL EtOH, mix on orbital shaker for 5 min, add 200 µL water and 500 µL hexane, mix for 10 min, centrifuge at 2000 g for 10 min at 17°, remove 300 µL upper organic layer. Re-extract with 300 µL hexane, mix for 10 min, centrifuge at 4000 g for 10 min at 17°, remove 300 µL upper organic layer. Combine the organic layers, and evaporate them to dryness under vacuum in 15 min. Reconstitute the residue with 300 µL MeOH:EtOH:hexane 88:10:2, vortex for 10 min, inject a 40 µL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 3 µm Adsorbosphere HS C18 + 150 × 4.6 3 µm Adsorbosphere HS C18 in series

Mobile phase: Gradient. A was MeCN:MeOH 60:40 containing 0.05% acetic acid. B was MeCN:MeCN:dichloromethane 45.6:30.4:24 containing 0.04% acetic acid. A:B 100:0 for 7 min then 0:100 for 10.4 min (step gradient), re-equilibrate at initial conditions for 5.6 min.

Column temperature: 37

Flow rate: 0.9

Injection volume: 40

Detector: UV 450

CHROMATOGRAM

Retention time: 16.5

Internal standard: tocol (UV 292) (10.1), echinenone (UV 450) (12.8)

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: canthaxanthine (UV 473), α-carotene (UV 450), β-cryptoxanthine (UV 450), lutein (UV 450), lycopene (UV 473), vitamin A (UV 325), vitamin E (UV 292), zeaxanthin (UV 450), nonidentified carotenoids

KEY WORDS

serum

REFERENCE

Steghens,J.-P.; van Kappel,A.L.; Riboli,E.; Collombel,C. Simultaneous measurement of seven carotenoids, retinol and α-tocopherol in serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *694*, 71–81.

SAMPLE

Matrix: blood

Sample preparation: Add 1 mL 1 µg/mL retinyl palmitate, 1 µg/mL retinyl palmitate, and 25 µg/mL α-tocopheryl acetate in EtOH to 1 mL serum or plasma while continuously vortexing, add 3 mL hexane, vortex for 2 min, centrifuge at 2500 g for 2 min, remove the upper phase, add 2 mL hexane to the lower layer, repeat extraction. Combine the upper layers and evaporate them to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 µL mobile phase, inject a 40 µL aliquot.

HPLC VARIABLES
Guard column: C18 (Waters)
Column: 5 μm Biophase ODS C18 (Bioanalytical Systems)
Mobile phase: MeCN:chloroform:isopropanol:water 78:16:3.5:2.5
Flow rate: 2
Injection volume: 40
Detector: UV 460 (UV 292 for tocopherol)

CHROMATOGRAM
Retention time: 16.46
Internal standard: retinyl acetate (3.07), retinyl palmitate (18.66), α-tocopheryl acetate (8.33)

OTHER SUBSTANCES
Extracted: vitamin A (retinol), vitamin E (α-tocopherol), gamma-tocopherol, α-carotene, lycopene, cryptoxanthin

KEY WORDS
serum; plasma

REFERENCE
Kaplan,L.A.; Miller,J.A.; Stein,E.A.; Stampfer,M.J. Simultaneous, high-performance liquid chromatographic analysis of retinol, tocopherols, lycopene, and α- and β-carotene in serum and plasma, *Methods Enzymol.*, **1990**, *189*, 155–167.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Serum + 25 μL 80 μg/mL tocol in EtOH + 250 μL 20 μg/mL BHT (butylated hydroxytoluene) in EtOH + 1.5 mL hexane, vortex for 1 min, remove 1 mL of upper layer, add 500 μL hexane, vortex for 1 min, remove 300 μL of upper layer. Combine the hexane extracts, evaporate to dryness under a stream of inert gas. Reconstitute in 250 μL 20 μg/mL BHT in EtOH, sonicate, centrifuge if necessary, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Vydac 201TP54 (wide pore, polymerically bonded C18)
Mobile phase: Gradient. A was MeOH:n-butanol:water 75:10:15 containing 50 mM ammonium acetate, pH 5.5. B was MeOH:n-butanol:water 88:10:2 containing 50 mM ammonium acetate, pH 5.5. A:B 100:0 for 3 min, to 0:100 over 15 min, maintain at 0:100 for 17 min
Injection volume: 25
Detector: UV 325 for 7 min, UV 295 for 13 min, UV 450 for 14 min or E, glassy carbon electrode, Ag/AgCl reference electrode +1050 mV for retinol, +900 mV for tocol, +750 mV for α-tocopherol, +700 mV for β-carotene

CHROMATOGRAM
Retention time: 31
Internal standard: tocol (13)
Limit of detection: 2.1 μg/mL (E), 29 μg/mL (UV)

OTHER SUBSTANCES
Extracted: vitamin A (retinol), vitamin E (α-tocopherol), gamma-tocopherol, lutein, zeaxanthin, cryptoxanthin, α-carotene, 9-cis-β-carotene

KEY WORDS
serum

REFERENCE
MacCrehan,W.A. Determination of retinol, α-tocopherol, and β-carotene in serum by liquid chromatography, *Methods Enzymol.*, **1990**, *189*, 172–181.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 100 μL EtOH + 100 μL α-tocopheryl acetate in EtOH, vortex for 5 s, add 500 μL hexane, vortex for 2 min, centrifuge at 700 g for 5 min. Remove 250 μL of the hexane layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, mix for 2 min, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 10 μm Spheri-10 RP18
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:dichloromethane:MeOH 70:20:10
Flow rate: 1.2
Injection volume: 50
Detector: UV 325 for 3.5 min, UV 291 for 4.5 min, UV 450 for 6 min

CHROMATOGRAM
Retention time: 11.79
Internal standard: α-tocopheryl acetate (6.30)
Limit of detection: 50 nM

OTHER SUBSTANCES
Extracted: vitamin A, vitamin E

KEY WORDS
protect from light; serum

REFERENCE
Arnaud,J.; Fortis,I.; Blachier,S.; Kia,D.; Favier,A. Simultaneous determination of retinol, α-tocopherol and β-carotene in serum by isocratic high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *572*, 103–116.

SAMPLE
Matrix: blood
Sample preparation: 2.5 mL Plasma + 2.5 mL 18 ng/mL IS1 and 10 ng/mL IS2 in EtOH, shake vigorously for 20 s, centrifuge at 1200 g for 5 min, add 5 mL diethyl ether, shake vigorously, centrifuge for 5 min, extract twice more with 5 mL ether. Combine ether layers, wash with 15 mL 5% NaCl, dry over sodium sulfate, evaporate to dryness under vacuum at 35°. Dissolve residue in 1-2 mL dichloromethane, filter (0.45 μm). Evaporate to dryness under a stream of nitrogen, make up to 100 μL with MeCN:MeOH:dichloromethane:hexane 45:10:22.5:22.5, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Spheri-5-C18 (Brownlee)
Column: 250 × 4.6 5 μm Microsorb C18 (Rainin)
Mobile phase: Gradient. MeCN:MeOH:dichloromethane:hexane 85:10:2.5:2.5 for 10 min then to 45:10:22.5:22.5 over 30 min, re-equilibrate for 15 min
Flow rate: 0.7
Injection volume: 20
Detector: UV 470

CHROMATOGRAM
Retention time: 34

Internal standard: IS1 ethyl β-apo-8'-carotenate (18), IS2 (3R)-8'-apo-β-carotene-3,8'-diol (5)

OTHER SUBSTANCES
Extracted: carotenoids, vitamin A (retinol), vitamin E (α-tocopherol)

KEY WORDS
plasma; handle under yellow lights

REFERENCE
Khachik,F.; Beecher,G.R.; Goli,M.B.; Lusby,W.R.; Smith,J.C.,Jr. Separation and identification of carotenoids and their oxidation products in the extracts of human plasma, *Anal.Chem.*, **1992**, *64*, 2111–2122.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum or plasma + 200 μL 25 μg/mL tocopheryl acetate in EtOH, vortex, add 400 μL butanol:ethyl acetate 50:50, mix for 1 min, add 20 mg sodium sulfate, vortex for 1 min, let stand at -20° for 20 min, centrifuge at 15000 g for 2 min, inject a 10 μL aliquot of the upper organic layer.

HPLC VARIABLES
Guard column: 5 μm C18
Column: 110 × 4.7 5 μm Partisphere 5 C18 (Whatman)
Mobile phase: MeOH:butanol:water 89.5:5:5.5
Column temperature: 45
Flow rate: 1.5
Injection volume: 10
Detector: UV 340 for 3 min, UV 290 for 1.5 min, UV 280 for 10.5 min, UV 450 for 7 min

CHROMATOGRAM
Retention time: 20.1
Internal standard: tocopheryl acetate (5.3)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: α-carotene, lycopene, δ-tocopherol, gamma-tocopherol, vitamin A, vitamin E, xanthophyll

KEY WORDS
serum; plasma; protect from light

REFERENCE
Lee,B.L.; Chua,S.C.; Ong,H.Y.; Ong,C.N. High-performance liquid chromatographic method for routine determination of vitamins A and E and β-carotene in plasma, *J.Chromatogr.*, **1992**, *581*, 41–47.

SAMPLE
Matrix: blood
Sample preparation: Dilute 1 mL serum 0.5-5 times with saline. Add 1 mL EtOH to 1 mL diluted serum dropwise while vortexing, add 1.5 mL n-heptane, vortex for 1 min, centrifuge at 3000 rpm (Labofuge) for 15 min. Remove 1.3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 40 μL MeCN:THF 50:50, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 200 × 2.1 5 μm ODS Hypersil
Mobile phase: MeCN:water:THF 81.3:5.7:13

Column temperature: 40
Flow rate: 0.4
Injection volume: 5
Detector: UV 450

CHROMATOGRAM
Retention time: 14.230
Limit of detection: 1 ng

OTHER SUBSTANCES
Extracted: Vitamin A (retinol), Vitamin E (α-tocopherol), probucol, gamma-tocopherol, lycopene, α-carotene, metabolites

KEY WORDS
serum

REFERENCE
Schäfer Elinder,L.; Walldius,G. Simultaneous measurement of serum probucol and lipid-soluble antioxidants, *J.Lipid Res.*, **1992**, *33*, 131–137.

SAMPLE
Matrix: blood
Sample preparation: 20-500 μL Serum + 2 volumes EtOH + 1 mL ethyl acetate + 4-7 μL of a solution containing 16 mg/mL tocopheryl acetate, 2-3 μg/mL canthaxanthin, and 10 μg/mL retinoic acid, vortex for 30 s, centrifuge for 30 s, extract the pellet twice with 0.5-1 mL portions of ethyl acetate, extract the pellet with 0.5-1 mL hexane. Combine the supernatants, add 500 μL water, vortex, centrifuge. Remove the upper organic layer and evaporate it to dryness under a stream of argon, reconstitute the residue in 100 μL MeOH:dichloromethane 2:1, inject a 10-90 μL aliquot.

HPLC VARIABLES
Guard column: C18 (Upchurch)
Column: 300 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeCN:dichloromethane:MeOH:1-octanol 90:15:10:0.1
Flow rate: 1
Injection volume: 10-90
Detector: UV 450

CHROMATOGRAM
Retention time: 21
Internal standard: tocopheryl acetate, canthaxanthin, retinoic acid

OTHER SUBSTANCES
Extracted: carotenoids, vitamin A (UV 325), vitamin E (UV 290)

KEY WORDS
protect from light; serum

REFERENCE
Barua,A.B.; Kostic,D.; Olson,J.A. New simplified procedures for the extraction and simultaneous high-performance liquid chromatographic analysis of retinol, tocopherols and carotenoids in human serum, *J.Chromatogr.*, **1993**, *617*, 257–264.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum or plasma + 500 μL EtOH containing 4.27 μM retinyl acetate and 0.31 μM echinenone, rotamix for 30 s, add 2 mL n-hexane, rotamix for 30 s,

centrifuge at 2000 g for 2 min, repeat extraction with 2 mL n-hexane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 50 μL THF, make up to 200 μL with EtOH, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 5 μm Spherisorb ODS1
Column: 250 × 4.6 5 μm Spherisorb ODS1
Mobile phase: Gradient. A was MeCN:MeOH 20:80 containing 100 mM ammonium acetate. B was 100 mM ammonium acetate in water. A:B from 90:10 to 100:0 over 12 min, maintain at 100:0 for 10 min, re-equilibrate at initial conditions for 5 min
Flow rate: 2
Injection volume: 50
Detector: UV 325 for 7.5 min, UV 292 for 5.5 min, then UV 450

CHROMATOGRAM
Retention time: 19.50
Internal standard: retinyl acetate (5.96), echinenone (15.15)
Limit of detection: 0.13 μM

OTHER SUBSTANCES
Extracted: cryptoxanthin, lutein, lycopene, vitamin A, vitamin E

KEY WORDS
plasma; protect from light; serum

REFERENCE
Zaman,Z.; Fielden,P.; Frost,P.G. Simultaneous determination of vitamins A and E and carotenoids in plasma by reversed-phase HPLC in elderly and younger subjects, *Clin.Chem.*, **1993**, *39*, 2229–2234.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma or serum + 200 μL 850 ng/mL retinyl acetate in EtOH, mix for 1 min, add 1 mL 0.4 g/L BHT (2,6-di-tert-butyl-4-methylphenol) in n-hexane, shake on a mechanical shaker for 10 min, centrifuge at 2000 g for 5 min, remove 800 μL of the supernatant, evaporate to dryness at 40° under a stream of nitrogen, reconstitute in 100 μL MeCN:THF:MeOH 68:22:7, inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Lichrosorb RP18
Column: 250 × 4.6 5 μm Nucleosil 100-5 C18
Mobile phase: MeCN:THF:MeOH 68:22:7 made up to 100 with 1% ammonium acetate
Flow rate: 1.5
Injection volume: 15
Detector: UV 325 for 3 min, UV 450 for 1.9 min, UV 290 for 2.5 min, UV 470 for 4.6 min, UV 450 for 3 min, then UV 325 for rest of run

CHROMATOGRAM
Retention time: 13
Internal standard: retinyl acetate (2.7)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: vitamin A (retinol), vitamin E (α-tocopherol), lutein, lycopene, α-carotene, zeaxanthin, trans β-carotene, δ-tocopherol

KEY WORDS
plasma; serum; protect from sunlight

REFERENCE
Bui,M.H. Simple determination of retinol, α-tocopherol and carotenoids (lutein, all-*trans*-lycopene, α- and β-carotenes) in human plasma by isocratic liquid chromatography, *J.Chromatogr.B*, **1994**, *654*, 129–133.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL nonapreno-β-carotene and retinyl butyrate in EtOH, vortex for 10 s, add 1 mL hexane, vortex for 30 s, centrifuge at 1500 g for 5 min. Remove 900 μL of the hexane layer and evaporate it to a waxy or glassy consistency (not dryness) under vacuum, dissolve in 100 μL EtOH, add 100 μL MeCN, vortex, filter (0.45 μm), inject a 30 μL aliquot.

HPLC VARIABLES
Column: 1540 × 4.6 5 μm Ultramex C18 (Phenomenex)
Mobile phase: MeCN:EtOH 50:50 containing 0.1 mL/L diethylamine
Column temperature: 29
Flow rate: 0.9
Injection volume: 30
Detector: UV 450

CHROMATOGRAM
Retention time: 8.10
Internal standard: nonapreno-β-carotene (9.5, UV 450), retinyl butyrate (3.5, UV 300)
Limit of detection: 13 nM

OTHER SUBSTANCES
Extracted: vitamin A (retinol), vitamin E (α-tocopherol), lutein, zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, retinyl linoleate, retinyl oleate, retinyl palmitate, retinyl stearate

KEY WORDS
serum; use gold fluorescent lamps; hold sample at 4° before injection

REFERENCE
Sowell,A.L.; Huff,D.L.; Yeager,P.R.; Caudill,S.P.; Gunter,E.W. Retinol, α-tocopherol, lutein/zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, trans-β-carotene, and four retinyl esters in serum determined simultaneously by reversed-phase HPLC with multiwavelength detection, *Clin.Chem.*, **1994**, *40*, 411–416.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 2.5 mL EtOH, mix for 5 min, add 5 mL n-hexane, mix vigorously, centrifuge at 2000 g for 5 min, repeat extraction with 3 mL n-hexane. Combine the n-hexane layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in dichloromethane, inject an aliquot.

HPLC VARIABLES
Guard column: μBondapak C18 Guard-Pak + 50 mm long C18 ODS(4) (Shimadzu)
Column: 250 × 4.6 5 μm Vydac 201 TP 54 C18
Mobile phase: MeOH:MeCN 90:10 (Every 100 injections wash column with MeOH:MeCN: dichloromethane 8:1:1.)
Flow rate: 1
Detector: UV 451

CHROMATOGRAM
Retention time: 18.5 (all-trans), 20.7 (9-cis), 21.8 (15-cis), 19.7 (9,15-dicis)

OTHER SUBSTANCES
Extracted: vitamin A (UV 324), vitamin E (UV 291)

KEY WORDS
serum

REFERENCE
Ben-Amotz,A. Simultaneous profiling and identification of carotenoids, retinols, and tocopherols by high performance liquid chromatography equipped with three-dimensional photodiode array detection, *J.Liq.Chromatogr.*, **1995**, *18*, 2813–2825.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL 650 ng/mL tocopheryl acetate in MeOH, vortex for 30 s, add 200 μL n-hexane, shake for 15 min, centrifuge at 3000 rpm for 10 min. Remove 120 μL of the hexane layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 20 μL dichloromethane, add 100 μL MeCN:MeOH 50:50, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:dichloromethane 50:45:5
Flow rate: 0.7
Injection volume: 50
Detector: UV 450

CHROMATOGRAM
Retention time: 18.4 (all trans), 19.5 (15,15'-cis)
Internal standard: tocopheryl acetate (F ex 295 em 330) (9.3)

OTHER SUBSTANCES
Extracted: vitamin A (F ex 325 em 480), vitamin E (F ex 295 em 330), γ-tocopherol (F ex 295 em 330), retinyl palmitate (F ex 325 em 480), α-carotene

KEY WORDS
serum

REFERENCE
Yakushina,L.; Taranova,A. Rapid HPLC simultaneous determination of fat-soluble vitamins, including carotenoids, in human serum, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 715–718.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 100 μL Plasma + 100 μL 0.9% NaCl + 200 μL MeOH, vortex for 30 s, let stand for 10 min, add 400 μL chloroform, vortex for 4 min. Remove the chloroform layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in MeOH, inject an aliquot. Tissue. Homogenize (liver with Mikro-dismembrator II in liquid nitrogen; other tissue with Ultra-Turrax) tissue with 3 mL 1% acetic acid containing 1 mg/mL ascorbic acid and 10 mM EDTA, add 2 mL MeOH, vortex for 30 s, let stand for 10 min, add 4 mL chloroform, vortex for 4 min. Remove the chloroform layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in MeOH, inject an aliquot.

HPLC VARIABLES
Column: 125 × 4.6 3 μm Hypersil ODS
Mobile phase: MeCN:dichloromethane:MeOH:water 70:10:15:5

Flow rate: 0.5 for 13 min, to 1 over 1 min, maintain at 1 for 10 min, to 1.5 over 1 min, maintain at 1.5 for 21 min, to 2 over 1 min, maintain at 2 for 10 min, return to 0.5 over 1 min, maintain at 0.5 for 2 min.
Injection volume: 50
Detector: UV 445

CHROMATOGRAM
Retention time: 39
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: vitamin A (UV 350), vitamin E (UV 292)

KEY WORDS
rat; protect from light; liver; plasma; lung

REFERENCE
Van Vliet,T.; Van Schaik,F.; Van Schoonhoven,J.; Schrijver,J. Determination of several retinoids, carotenoids and E vitamers by high-performance liquid chromatography. Application to plasma and tissues of rats fed a diet rich in either β-carotene or canthaxanthin, *J.Chromatogr.*, **1991**, *553*, 179–186.

SAMPLE
Matrix: cheese
Sample preparation: 500 mg Cheese + 2 mL 60% KOH + 2 mL 95% EtOH + 1 mL 1% NaCl + 5 mL 6% pyrogallol in EtOH, flush tube with nitrogen, seal, heat at 70° for 30 min, cool in ice water, add 15 mL 1% NaCl, extract twice with 15 mL portions of n-hexane:ethyl acetate 90:10. Combine the organic layers and evaporate them to dryness, dissolve the residue in 2 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere Si
Mobile phase: Gradient. A was n-hexane:isopropanol 99:1. B was n-hexane. A:B 50:50 for 7 min; to 90:10 over 4 min, maintain at 90:10 for 7 min, to 50:50 over 1 min, maintain at 50:50 for 4 min. (About every 100 injections recondition column with 50 mL dichloromethane, 50 mL isopropanol, and 50 mL dichloromethane.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 450 (β-carotene) and F ex 325 em 475 for 3.5 min, ex 280 em 475 for 10.5 min, ex 325 em 475 for 9 min (others)

CHROMATOGRAM
Retention time: 2
Limit of detection: 0.16 ng

OTHER SUBSTANCES
Extracted: vitamin E (α-tocopherol), vitamin A (all-trans-retinol), β-tocopherol, gamma-tocopherol, δ-tocopherol, 13-cis-retinol

KEY WORDS
normal phase; cheese

REFERENCE
Panfili,G.; Manzi,P.; Pizzoferrato,L. High-performance liquid chromatographic method for the simultaneous determination of tocopherols, carotenes, and retinol and its geometric isomers in Italian cheeses, *Analyst*, **1994**, *119*, 1161–1165.

SAMPLE
Matrix: food

Sample preparation: Dissolve 10 g margarine in 50 mL dichloromethane, add 3 g anhydrous magnesium sulfate, let stand for 2 h with frequent agitation, filter (fritted glass), make up filtrate to 100 mL with dichloromethane, inject a 250 μL aliquot on to four μStyragel 100 Å GPC columns (Waters) in series, elute with dichloromethane at 1 mL/min, monitor at 313 nm, collect the fraction corresponding to β-carotene (at 23.5-26.5 min) and evaporate it to dryness, reconstitute with mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax C18
Mobile phase: MeCN:dichloromethane:MeOH 70:30:0.2
Flow rate: 1
Injection volume: 100
Detector: UV 436

CHROMATOGRAM

Retention time: 15

OTHER SUBSTANCES

Simultaneous: vitamin A palmitate

KEY WORDS

margarine

REFERENCE

Chase,G.W.,Jr.; Akoh,C.C.; Eitenmiller,R.R.; Landen,W.O. Liquid chromatographic method for the concurrent analysis of sucrose polyester, vitamin A palmitate, and β-carotene in margarine, *J.Liq.Chromatogr.*, **1995**, *18*, 3129–3138.

SAMPLE

Matrix: fruit, juice, oil, vegetables
Sample preparation: Squash, peach. Homogenize (Waring blender) 10-30 g finely chopped squash or peach with 100 mL acetone and 10 g Hyflo supercel for 3 min, filter. repeat extraction of the solids until all the pigment was removed, extract with petroleum ether, wash the organic layer with water, pass it through a short column containing anhydrous sodium sulfate, concentrate under reduced pressure, inject an aliquot. Orange juice, palm tree oil. Homogenize (Waring blender) 10-30 g orange juice or palm tree oil with 100 mL acetone and 10 g Hyflo supercel for 3 min, filter. repeat extraction of the solids until all the pigment was removed, extract with petroleum ether, add 10% KOH in MeOH, let stand overnight, wash the organic layer with water, pass it through a short column containing anhydrous sodium sulfate, concentrate under reduced pressure, inject an aliquot.

HPLC VARIABLES

Column: 250 × 2.2 $Ca(OH)_2$ laboratory packed ($Ca(OH)_2$ from Nakarri Chemicals.)
Mobile phase: Isooctane (squash, palm tree oil) or Gradient. Isooctane:acetone from 100:0 to 80:20 over 1 h. (orange juice, peach)
Flow rate: 0.5
Injection volume: 10
Detector: UV 450

CHROMATOGRAM

Retention time: 10 (13-cis), 15 (trans), 22 (9-cis) (isocratic mobile phase)

KEY WORDS

squash; peach; orange juice; palm tree oil; normal phase

REFERENCE

Carvalho,C.R.L.; Carvalho,P.R.N.; Collins,C.H. High-performance liquid chromatographic determination of the geometrical isomers of β-carotene in several foodstuffs, *J.Chromatogr.A*, **1995**, *697*, 289–294.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 2-25 μL aliquot of a solution on mobile phase.

HPLC VARIABLES
Column: 250 × 4.6 5 μm C30 silica (Anal.Chem. 1994, 66, 1667)
Mobile phase: MTBE:MeOH 89:11
Flow rate: 1
Injection volume: 2-25
Detector: UV 453

CHROMATOGRAM
Retention time: 50 (all-trans)

OTHER SUBSTANCES
Simultaneous: isomers

REFERENCE
Emenhiser,C.; Sander,L.C.; Schwartz,S.J. Capability of a polymeric C_{30} stationary phase to resolve *cis-trans* carotenoid isomers in reversed-phase liquid chromatography, *J.Chromatogr.A*, **1995**, *707*, 205–216.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 3 μm Adsorbosphere-HS C18
Mobile phase: MeCN:isopropanol:MeOH 60:30:10 containing 0.1% ammonium acetate
Flow rate: 1
Detector: UV 234, UV 295, UV 450

OTHER SUBSTANCES
Simultaneous: vitamin E

REFERENCE
Maitra,I.; Marcocci,L.; Droy-Lefaix,M.T.; Packer,L. Peroxyl radical scavenging activity of *Gingko biloba* extract EGb 761, *Biochem.Pharmacol.*, **1995**, *49*, 1649–1655.

SAMPLE
Matrix: vegetables
Sample preparation: Macerate vegetables (Waring blender), remove 5 g of this material and homogenize it with 10 mL EtOH (Biohomogenizer) for 3 min, add 2 mL pentane, homogenize for 2 min (purge homogenizer motor with nitrogen), centrifuge at 7000 g for 3 min, remove pentane layer, add 2 g NaCl and 5 mL water to lower layer, shake gently, add 8 mL pentane, shake vigorously for 2 min, centrifuge, remove pentane layer. Combine pentane layers and evaporate (if necessary) under a stream of nitrogen to reduce the volume, make up to 5 or 10 mL with pentane, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Vydac 201 TP54 C18
Mobile phase: MeOH:MeCN:dichloromethane:hexane 65:27:4:4
Flow rate: 1.5
Injection volume: 20
Detector: UV 450

CHROMATOGRAM
Retention time: 14

KEY WORDS
details for SFE also given; squash; broccoli; carrots; collard greens; turnip; kale; mustard greens; zucchini

REFERENCE
Marsili,R.; Callahan,D. Comparison of a liquid solvent extraction technique and supercritical fluid extraction for the determination of α- and β-carotene in vegetables, *J.Chromatogr.Sci.*, **1993**, *31*, 422–428.

SAMPLE
Matrix: vegetables
Sample preparation: Blanch vegetables in water for 90 s. Stir 20 g homogenized and mashed vegetables with 50 mL ethyl ether for about 4 h, filter, extract the residue again. Combine the filtrates, add 5 mL 20% KOH in MeOH, mix, place in a refrigerator for about 12 h, add 100 mL water, shake vigorously, let stand for about 5 h. Remove the ether layer and concentrate it under vacuum, evaporate traces of ether with a stream of nitrogen, reconstitute with EtOH (?), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Wakosil-II 5C18 AR polymeric ODS (Wako Chemicals)
Mobile phase: THF:MeOH 10:90
Column temperature: 20
Flow rate: 1
Injection volume: 100
Detector: UV 450

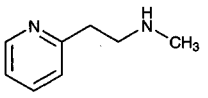
CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Extracted: α-carotene, β-cryptoxanthin, lutein, lycopene, zeaxanthin

KEY WORDS
carrots; tomatoes; pumpkins

REFERENCE
Jinno,K.; Lin,Y. Separation of carotenoids by high-performance liquid chromatography with polymeric and monomeric octadecylsilica stationary phases, *Chromatographia*, **1995**, *41*, 311–317.

Betahistine



Molecular formula: $C_8H_{12}N_2$
Molecular weight: 136.20
CAS Registry No.: 5638-76-6, 5579-84-0 (2HCL)
Merck Index: 1224
Lednicer No.: 2 279

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 3.155

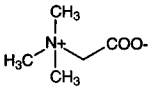
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Betaine



Molecular formula: $C_5H_{11}NO_2$

Molecular weight: 117.15

CAS Registry No.: 107-43-7, 590-46-5 (HCl), 590-47-6 (monohydrate), 93227-64-6 (sodium aspartate)

Merck Index: 1225

SAMPLE

Matrix: solutions

Sample preparation: Mix 600 µL solution with 60 µL IS solution, vortex, add a 550 µL aliquot to a 70 × 5 column of Dowex 1-X8 (OH^-) form in a Pasteur pipette, elute with 2 mL water, evaporate the eluate to dryness under a stream of air, reconstitute with 100 µL 2 mM N,N-diisopropylethylamine in MeCN, add 100 µL 5 mM 4'-bromophenacyl trifluoromethanesulfonate in MecN, vortex for 10 min, add 100 µL 10 mM hydroxyacetic acid N,N-diisopropylethylammonium salt in MeCN, vortex for 2 min, inject a 15 min aliquot. (Prepare 4'-bromophenacyl trifluoromethanesulfonate as follows. Add 8.8 g p-bromobenzoyl chloride in 40 mL dry ether over 20-30 min to 100 mmoles diazomethane stirred in an ice bath, stir in an ice bath for 8-9 h, let stand at room temperature for 3 h, evaporate the solvent under reduced pressure, recrystallize 4'-bromo-2-diazoacetophenone from ether/hexane (mp 123.5-124° d) (J.Am.Chem.Soc. 1951, 73, 5301). Condense 50 mL anhydrous sulfur dioxide in a flask fitted with a calcium sulfate drying tube, cool in a dry ice/acetone bath, add 2.25 g 4'-bromo-2-diazoacetophenone, stir for 5 min, add 900 µL anhydrous trifluoromethanesulfonic acid from a freshly opened bottle in one portion, stir for 15 min, remove the cooling bath, after 30 min use an ice/water bath to evaporate the solvent. Dissolve the residue in 100 mL boiling dichloromethane, treat twice with 5 g portions of decolorizing carbon, filter, evaporate the filtrate, recrystallize the residue from pentane:dichloromethane 80:20 to give 4'-bromophenacyl trifluoromethanesulfonate as colorless plates (mp 137-8°) (J. Chromatogr. 1984, 299, 365).)

HPLC VARIABLES

Guard column: 50 × 4 Co:Pell ODS

Column: 100 × 8 5 µm Radial-PAK C18

Mobile phase: MeCN:buffer 70:30 (Prepare by dissolving 70 mg sodium dodecyl sulfate, 140 mg $NaH_2PO_4.H_2O$, and 300 µL 3-dimethylamino-1-propanol in 150 mL water, adjusting pH to 6.5 with 85% phosphoric acid, and adding 350 mL MeCN.)

Flow rate: 5

Injection volume: 15

Detector: UV 254

CHROMATOGRAM

Retention time: 7

Internal standard: (4-bromophenyl)carboxymethyl (6-trimethylammonium)hexanoate (Add an aqueous solution of 6-(trimethylammonium)hexanoic acid (Cl^- salt, prepared by methylation of 6-aminohexanoic acid) to a column of Dowex 1-X8 (OH^- form), elute with 4 column volumes of water. Evaporate the eluate to dryness, dissolve the residue in DMF, add 1.1 equivalents of triethylamine, add 1.1 equivalents of 2,4'-dibromoacetophenone, stir at 40° for 3 h, add ethyl acetate. Collect the precipitate by filtration and recrystallize it from ethanol/acetone to obtain (4-bromophenyl)carboxymethyl (6-trimethylammonium)hexanoate.) (8)

Limit of quantitation: 10 µM

KEY WORDS

derivatization

REFERENCE

Minkler,P.E.; Ingalls,S.T.; Kormos,L.S.; Weir,D.E.; Hoppel,C.L. Determination of carnitine, butyrobetaine, and betaine as 4'-bromophenacyl ester derivatives by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *336*, 271–283.